UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

AEHA

TOXICOLOGICAL STUDY NO. 75-51-0497-91
PHASE 5
EFFECTS OF INGESTION OF ZINC NAPHTHENATE
ON THE REPRODUCTION FUNCTION OF RATS
JANUARY 1987 - FEBRUARY 1988

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This Study was conducted to determine the effects on reproduction, if any, of oral administration to rats of a candidate wood preservative, zinc naphthenate. Findings indicated that rats fed a diet of 0.5% zinc naphthenate experienced a significant weight loss. This weight loss had no effect on mating or viability of offspring over two generations of rats on study. Therefore, zinc naphthenate was not considered to be a reproductive hazard in rats under the conditions of this study. Recommendations provide for the use of appropriate personal protection when handling zinc naphthenate solutions.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO
ATTENTION OF

EXECUTIVE SUMMARY
TOXICOLOGICAL STUDY NO. 75-51-0497-91
PHASE 5
EFFECTS OF INGESTION OF ZINC NAPHTHENATE ON THE REPRODUCTION FUNCTION OF RATS
JANUARY 1987 - FEBRUARY 1988

1. PURPOSE. The U.S. Army is considering alternatives for the replacement of pentachlorophenol as a wood preservative for use on wooden ammunition packaging, pallets and skids. Increasing awareness of health hazards associated with the use of pentachlorophenol has prompted an investigation into other commercially available products. This study was conducted to determine the effects on reproduction, if any, of oral administration to rats of a candidate wood treatment, zinc naphthenate. Results will be used to establish potential health effects to personnel involved in handling and applying wood preservatives, or handling the treated materials.

2. ESSENTIAL FINDINGS. Rats fed a diet of 0.5 percent zinc naphthenate experienced a significant weight loss when compared to the lower dosage groups and control group. This weight loss had no effect on mating performance or viability of offspring over the two generations of rats on study.

3. CONCLUSIONS. Under the conditions of this study, zinc naphthenate was found not to be a reproductive hazard in rats. Concentrated solutions of zinc naphthenate are capable of producing toxic effects and are to be considered hazardous.

4. RECOMMENDATIONS. Use appropriate personal protection when handling all wood preservatives, including zinc naphthenate.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

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1. REFERENCES. See Appendix A for a list of references.

2. AUTHORITY.

a. Letter, AFPMB, Armed Forces Pest Management Board, Washington, DC, 25 September 1984, subject: Toxicology of Wood Preservatives.

b. Letter, DASG-PSP, Office of the Surgeon General, 3 May 1984, subject: Toxicological Hazards of Pentachlorophenol, Copper Naphthenate, Copper-8-Quinolinolate, and Zinc Naphthenate.

3. PURPOSE. This study was conducted to determine the effects, if any, of oral ingestion of zinc naphthenate on parental rat activities from mating through lactation and in growth and offspring development from conception through maturity over two generations of rats.

4. BACKGROUND.

a. The U.S. Army Armament Research and Development Command (ARDC) has taken action to eliminate reference to Federal Specification TT-W-572, Wood Preservative: Water-Repellent, from those specifications for which that command has custody. This specification covers generic types of pentachlorophenol, copper naphthenate, and copper-8-quinolinolate. In lieu of that reference, ARDC has listed several commercially available preservatives in each specification pertaining to treated wooden ammunition packing, pallets, and skids. Included in the listed preservatives are copper-8-quinolinolate, copper naphthenate, and zinc naphthenate.

Use of trademarked/company names does not imply endorsement by the U.S. Army but is intended only to assist in identification of a specific product.

b. U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) has conducted both a literature search and several acute toxicity studies on alternative wood preservatives. The literature search indicated that there was limited data available on the compounds to be studied (reference 1, Appendix A).

c. A further search of the data bases at the National Library of Medicine and the U.S. Environmental Protection Agency (EPA) confirmed the need to compile additional toxicity information for zinc naphthenate.

d. The results of other toxicity studies performed by this Agency on similar materials have been reported in previous Agency publications (references 2, 3 and 4, Appendix A).

5. MATERIALS.

a. Zinc naphthenate, $Zn(C_6H_5COO)_2$, CAS number 12001-85-3, is the reaction product of zinc oxide and naphthenic acids. It is a tarry, dark brown semi-solid with a pungent odor and contains 13.7 percent zinc. Synonyms for zinc naphthenate include zinc uversol and the zinc salt of naphthenic acid. The material used in this study was supplied by Mooney Chemicals Inc., Cleveland, Ohio, and was contained in two, plastic, 5-gallon containers. Upon receipt at this Agency, lot number P-17448 was tested and determined to be 97 percent pure.

b. Mazola® corn oil was used as a vehicle to dissolve zinc naphthenate and facilitate mixing in feed. Corn oil was also included in the control feed group to discount any toxic effects that may be caused by the vehicle.

c. Certified Rodent Ration used for this study was purchased from Zeigler Bros., Inc., Gardners, Pennsylvania. The ration arrived in the form of lab block and was ground to a uniform consistency using a Straub Grinding Mill, Model 4E, Philadelphia, Pennsylvania.

6. ANIMALS AND DOSAGE SELECTION.

a. A pilot study was first conducted using 72 Sprague Dawley COBS, CD rats (six groups, each of six male and six female) in accordance with reference 5, Appendix A, Standing Operating Procedure, Reproduction Study in Rats. Dosages for the study

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were calculated on a percent diet basis and were derived from expected food consumption and toxic signs seen in the acute oral studies. The concentration of zinc naphthenate in the feed ranged from 0.13 percent (1,300 ppm) in the low dosage group to 2.10 percent (21,000 ppm) in the high dosage group.

b. Sprague Dawley COBS, CD rats (120 female and 120 male), 5 weeks of age, were purchased from Charles River Laboratories of Wilmington, Massachusetts. Upon arrival, animals were randomly placed in hanging wire cages 41 cm wide, 36 cm deep, 17 cm high, three per cage, 40 cages per sex. Cages were numbered 1 through 80 with water and ground rat chow available *ad libitum*. On the following day, animals were weighed and assigned to four non-statistically different (by weight) dosage groups. All animals were then consecutively toe clipped for easy identification. Body weights and feed consumption were monitored during this pretreatment period.

c. After a 2-week quarantine, animals were judged to be fit by the veterinarian in charge and released for study start. All ground rat chow was discarded and replaced with treated feed of the appropriate dosage. Each dosage group consisted of 10 cages of male rats and 10 cages of female rats, 3 rats per cage. Cage labels and feed containers were color coded throughout the study to avoid administration of improper feed.

d. Three dosage levels of zinc naphthenate and a corn oil control were employed. The levels were as shown in Table 1. Dosages of zinc naphthenate were based on the previously conducted pilot study which indicated effects between 2,700 ppm and 5,000 ppm in both parental sexes and their offspring. Compound concentration in feed remained at a constant level throughout the entire study with no attempts to adjust for body weight gains.

TABLE 1. DOSAGE LEVELS

Group	Percent Zinc Naphthenate In Diet	PPM Zinc Naphthenate
Corn Oil Control	0.00	0
Low Dosage	0.05	500
Mid Dosage	0.10	1000
High Dosage	0.50	5000

e. Feed was prepared on a weekly basis by dissolving the appropriate amount of zinc naphthenate in corn oil with the aid of heat, while keeping the total volume of corn oil/zinc naphthenate for each dosage group constant. These solutions were, in turn, poured into ground rodent ration and thoroughly blended with a mechanical mixer. To ensure homogeneity and accuracy of the preparation, samples were extracted from each batch of feed and analyzed for zinc content by atomic absorption.

f. Water and treated feed were provided *ad libitum* to all animals. Efforts were made to maintain room temperature at 70°F (65-75), with a relative humidity of 50 percent (40-60). Artificial lighting was provided for 12 hours daily between 6:00 AM to 6:00 PM.

7. METHODS.

a. The 240 rats purchased from Charles Rivers Laboratories were designated as the P generation. After the 2-week holding period, rats were fed the zinc naphthenate diet for 10 weeks while sexes remained separate. Body weights and feed consumption were recorded three times per week during the exposure period. Animals were checked daily for toxic signs. Mating was begun on the eleventh week by pairing each female with a male of the same dosage group. Mating success was checked daily and was determined by the presence of sperm plugs on cage pads. When a positive mating was achieved, the females were removed from their wire cages and housed individually in polycarbonate boxes, where bedding material was provided. Animals not showing evidence of mating after 1 week were paired with a male of the same dosage group that had successfully mated. Again, after the second week, females failing to mate were given mates that were proven. Following the third week of mating, all remaining females for which there was no evidence of mating were also placed in polycarbonate boxes.

b. The P generation males were continued on treated feed until being submitted for necropsy on a staggered basis. Animals were euthanized by CO^2, examined grossly, and tissues removed (testes, epididymides, seminal vesicles, prostate, pituitary, liver, kidneys), for histopathologic examination in accordance with reference 6, Appendix A.

c. All P generation females were continually fed the treated diet during mating, gestation, and lactation. Dams were checked daily for new births or birthing complications.

d. Pups born to P dams were designated as the F_1 generation. Individual body weights, abnormalities, mortalities, and total litter weights were noted on day 0, 4, 7, 14, and 21 post partum. On day 4 post partum, litters were standardized by randomly culling to four males and four females each, or as close to equal numbers of sexes as permitted. Litters with less than eight pups on day 4 remained intact.

e. A table of random numbers was used to preselect 120 male and 120 female pups from the F_1 generation for continuation of the study. The remaining F_1 pups were submitted for gross only necropsy at weaning.

f. The P generation dams were also sacrificed by CO_2 at the time of weaning of the F_1 generation. All dams were examined grossly while the vagina, uterus, ovaries, pituitary gland, liver, and kidneys were removed for histopathologic examination.

g. The F_1 generation rats selected for study continuation were uniquely identified by toe clip and housed in the same manner as the P rats. To avoid severe inbreeding in the F_1's later in the study, P generation dam numbers were recorded for all F_1 rats kept for continuation. Administration of the appropriate feed dosages continued for 10 weeks following the last wean date, with daily observations, consumption, and body weights being monitored three times a week.

h. Mating of the F_1 rats was accomplished in the same manner as the P rats, with the exception of the care taken to avoid mating rats of the same litters. Again dosing continued through mating, gestation, and lactation of the F_1's.

i. Following the 3-week mating period, F_1 males were necropsied, taking sex and target organs for histopathologic examination. At weaning, five F_2 males and five F_2 female pups were selected from each group for removal of sex organs, liver, and kidneys for histopathology. The remaining F_2 pups were examined grossly while F_1 dams had organs taken for histopathologic examination.

j. In addition to the periodic sacrifices mentioned above, any animal dying spontaneously or for some reason removed from the study at an early date was submitted for gross necropsy. Gross necropsies consisted of the examination of all external surfaces, orifices, brain and spinal cord, thoracic, abdominal and pelvic cavities, and organs therein.

8. RESULTS.

a. <u>Pilot Study</u>. Male and female rats receiving 1 and 2 percent zinc naphthenate in their diet (10,000 ppm and 20,000 ppm) showed reduced body weights following 4 weeks of compound

administration. Both sexes of rats in these groups also became lethargic and experienced urogenital staining from compound consumption. Mating of rats within their respective groups indicated the 2 percent zinc naphthenate group required twice the mating time as did the other five dosage groups. Litters born of the highest two dosage groups (one and two percent) experienced reduced birth numbers and reduced total birth weights. At the time of weaning, offspring numbers and weights for these high-dosage group litters were greatly reduced when compared to litters of lower dosage groups and controls.

b. P (Parental) Generation.

(1) The P generation female rats in the 0.5 percent group (5,000 ppm) experienced significantly reduced body weights by week 3 of compound administration, and continued through week 10 (Appendix B). Appendix C shows the depressed weight gains experienced in the high-dosage group, while the control, low, and mid-dosage groups gained at similar rates.

(2) Male rats fed a diet of 5,000 ppm zinc naphthenate demonstrated significantly decreased body weights by week 8 of the 10 week dosing period (Appendix D). Appendix E is a graphic representation of male body weights prior to breeding, showing the gradual effects of the 0.5 percent zinc naphthenate diet on body weights.

(3) Weekly monitoring of feed consumption during the 10 week exposure period indicated all female rats ate similar portions, regardless of the dosage group (Appendix F). The same was also true for male rats, regardless of the zinc naphthenate concentration in the feed.

(4) Appendix G shows the actual zinc naphthenate consumption in mg/kg/day for rats in all dosage groups during the treatment period.

(5) Mating was attempted with 30 rats in each dosage group as presented in Appendix H. The fertility index for all dosage groups was comparable, with pregnancies resulting in between 79 and 86 percent of all rats. All dams produced live litters, with pups in all dosage groups remaining viable through lactation.

(6) A comparison of litter size using the Student "t" probability test showed a significant reduction in the high-dosage group when compared with that of the control group (Appendix I). Due to the fewer numbers of pups born to the 0.5 percent group dams, litter birth weights were also decreased to a significant degree. However, individual pup weights in this

high-dosage group were similar to those of the control group, while the pup weights of the low-dosage group were found to be slightly higher.

(7) At weaning, pups of the 0.5 percent dosage group had significantly lower body weights than the control, 0.05 percent, or 0.1 percent dosage groups.

(8) Male rats in the 0.5 percent group appeared to become lethargic after week 9 of the dosing period. Alopecia was prevalent in the mid- and high-dosage animals of both sexes, and their offspring.

(9) One male rat (No. 581) from the 0.1 percent dosage group was found cannibalized while the rats were group housed, prior to mating. Fighting was the apparent cause of death.

(10) Dam No. 496 of the 0.5 percent dosage group delivered all dead pups as a result of her water bottle not being filled over the weekend period prior to her delivery. As a result, the dam was removed from the study and was not included in any statistical determinations.

(11) Histopathological examination of P generation rat tissues revealed compound-related lesions in the kidneys of male rats of the 0.5 percent dosage group. These lesions consisted of accumulations of amorphous to slightly granular, lightly eosinophilic material in the lumina of renal tubules, particularly near the corticomedullary junction. The term "nephrosis" was used to describe the lesions which were considered microscopically distinct from the intraluminal accumulations of homogeneous proteinic material associated with the spontaneous degenerative nephropathy syndrome of rats. "Epithelial regeneration" of the renal tubules was evident, which may be associated with a variety of degenerative processes, especially the spontaneous degenerative nephropathy syndrome, mentioned above. However the increased incidence of this regeneration observed in the high-dosage rats suggests a compound-related effect. These lesions were not so advanced as to expect a clinically detectable effect on renal function. Lower concentrations of compound did not produce these effects, and all other findings were considered incidental (reference 7, Appendix A).

c. F, Generation.

(1) Body weights of female rats in the 0.5 percent dosage group were significantly depressed from the first week of the dosing period through week 10, when compared to the control group weights (Appendix J). Elevated body weights were observed in the 0.05 percent group from weeks 5 through 10. A graphic representation of these body weights is shown in Appendix K.

(2) As with the female rats, male rats in the 0.5 percent dosage group experienced significantly reduced lowered body weights throughout the pre-mating period (Appendix L). Again, male rats in the 0.05 percent group exceeded the body weights of the control and mid-dosage groups during weeks 7 through 10. Appendix M presents this data in graphic form.

(3) Feed consumption figures during the dosing period revealed that all groups of rats, both male and female, ate similar portions of chow, and were not influenced by compound concentrations (Appendix N).

(4) Appendix O presents actual zinc naphthenate consumption in mg/kg/day for the F_1 generation during the pre-mating period.

(5) Mating time for all F_1 rats proved to be of a longer duration than the P generation, with an average of 4.8 days before signs of mating were observed (Appendix P). Mating was attempted with 29-30 rats in each dosage group. Pregnancies resulted in 67 percent and 60 percent of the control and 0.05 percent groups, respectively, while 93 percent and 97 percent of the 0.1 percent and 0.5 percent animals became pregnant. All pregnancies resulted in live litters with the exception of one 0.5 percent dam (No. 238), which produced all stillborn fetuses after being unable to deliver at the predicted time. Pup survivability was similar for all dosage groups, through lactation.

(6) Appendix Q is a summary of the F_2 pups produced by the F_1 dams. Litter size and weights at birth were alike for all dosage groups. At weaning, individual pup weights as well as litter weights were significantly lowered in the 0.5 percent group.

(7) Dam No. 207 of the 0.1 percent dosage group was removed from study due to a delivery during the pre-mating period. Mis-sexing and group housing of rats resulted in an early sacrifice for this dam.

(8) Dam No. 146, a control group animal, was found dead at approximately 17 days of pregnancy, and was submitted for necropsy. No apparent reason could be determined for the cause of death.

(9) One control group dam (No. 140) gave birth to a pup suffering from craniorachischisis partialisis, which was considered to be a spontaneous abnormality, not associated with the test regimen.

(10) Histopathological examination of tissues from the F_1 generation rats again showed "nephrosis" and "tubular regeneration" in the kidneys of a small number of male rats which was apparently associated with compound administration. All other findings were considered incidental or associated with spontaneous disease complexes of rats (reference 8, Appendix A).

9. DISCUSSION.

(1) The P generation rats fed a diet of 0.5 percent zinc naphthenate exhibited depressed body weights even though they consumed comparable amounts of chow when compared to other dose groups. Ingestion of large amounts of zinc has been reported to cause gastroenteritis, resulting in growth retardation (reference 9, Appendix A). Pups produced by dams in this group were of normal size at birth but, by the time of weaning, they too showed significantly lowered body weights due to maternal stress during lactation. The reduced litter size at birth observed in this group may also be attributed to maternal stress experienced during pregnancy.

(2) Lethargy, observed among the 0.5 percent males, did not have an effect on their breeding performance. All groups of P generation rats had similar fertility indices, within historical limits. A differentiation was seen in the mating success of the F_1 generation rats between the 500 ppm and 1,000 ppm dosages. Rats in the control and low-dosage groups had significantly less mating success than observed in the P generation, while rats in the mid- and high-dosage groups outperformed those observed in the initial breeding. Diets of corn oil over two generations may have influenced breeding performance in the control and low-dosage groups. Fertility indicies of the mid- and high-dosage groups appear to have been effected by the high concentrations of zinc in their diet. High levels of zinc are commonly found in the male reproductive system and the epididymis, prostate, and testes of various species.

(3) The increase in pup size (F_1) observed at birth in the low-dosage group P generation dams may be attributed to the fact that dams of this dosage group were slightly (but not significantly) larger at the time of breeding. This fact also attributed to the significant body weight increases observed in the mature male and female rats of the F_1 generation, when compared to the control groups.

10. CONCLUSIONS.

(1) The continuous diets of zinc naphthenate employed in this study produced no adverse effects on the reproductive function of rats over two generations.

(2) Pup survivability from gestation through lactation was unaffected by the regimen of zinc naphthenate.

(3) Reduced body weights, observed at the 0.5 percent (5,000 ppm) dosage level in both male and female rats, indicated the onset of paternal and maternal toxicity, which would occur before a reproductive hazard would be observed.

11. QUALITY ASSURANCE. This study was conducted under the surveillance of the Analytical Quality Assurance Division, AEHA, in accordance with the guidelines listed in Appendix R.

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APPENDIX A

REFERENCES

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APPENDIX B

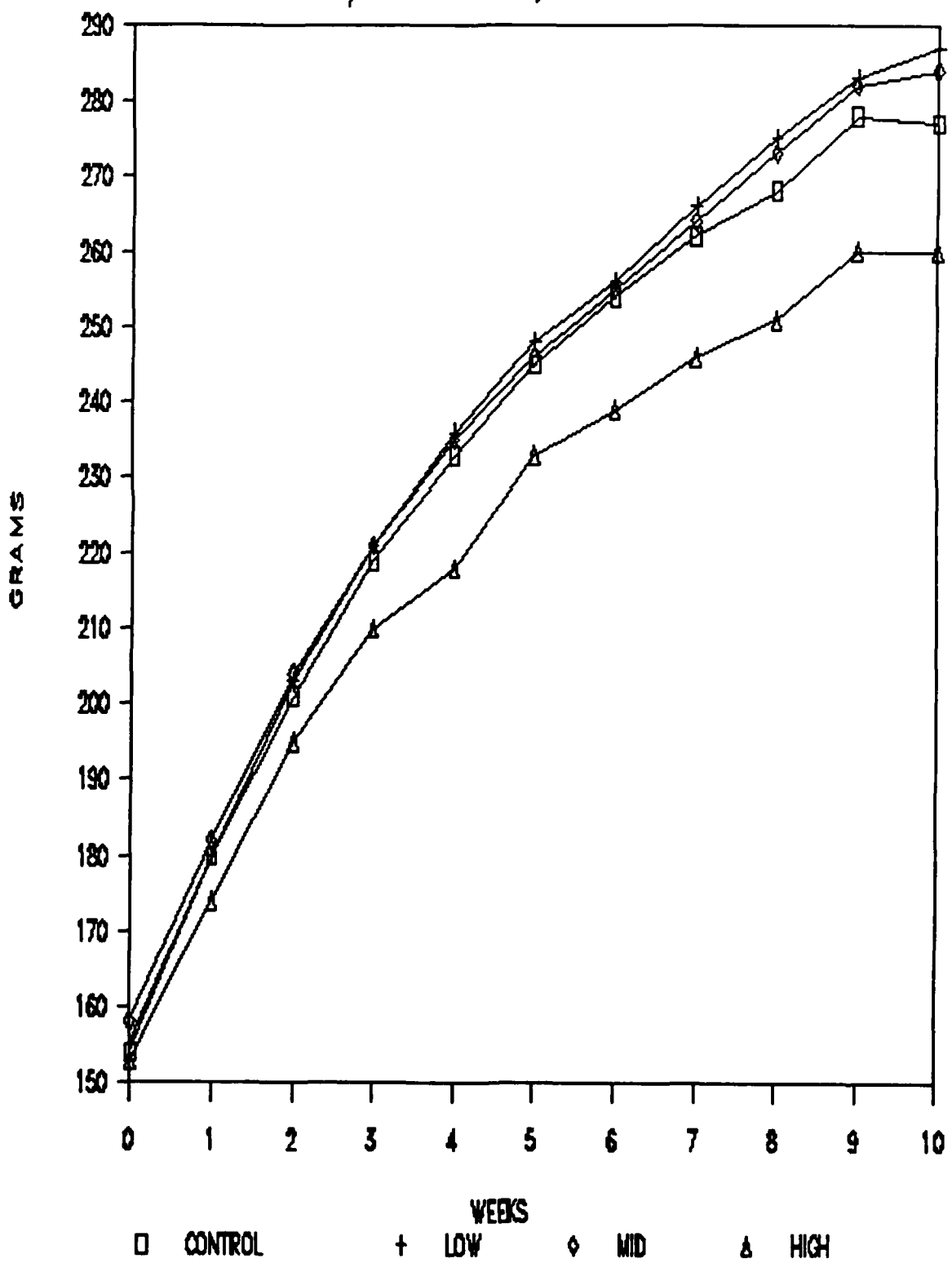
SUMMARY OF P FEMALE BODY WEIGHTS (gms)

WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
0	x	154	155	158	153
	SD	10	11	12	11
	t		0.21	1.23	0.59
	DF		58	58	58
1	x	180	180	182	174
	SD	11	13	16	12
	t		0.11	0.71	1.97
	DF		58	58	58
2	x	201	203	204	195
	SD	13	15	17	14
	t		0.69	0.72	1.57
	DF		58	58	58
3	x	219	221	221	210
	SD	15	18	20	15
	t		0.44	0.44	2.39*
	DF		58	58	58
4	x	233	236	235	218
	SD	15	21	22	25
	t		0.80	0.49	2.79*
	DF		58	58	58
5	x	245	248	246	233
	SD	16	22	23	18
	t		0.60	0.21	2.84*
	DF		58	58	58
6	x	254	256	255	239
	SD	19	23	24	18
	t		0.36	0.17	3.22*
	DF		58	58	58
7	x	262	266	264	246
	SD	19	24	26	18
	t		0.74	0.43	3.33*
	DF		58	58	58
8	x	268	275	273	251
	SD	20	25	27	19
	t		1.19	0.77	3.32*
	DF		58	58	58
9	x	278	283	282	260
	SD	22	28	27	21
	t		0.82	0.57	3.23*
	DF		58	58	58
10	x	277	287	284	260
	SD	23	32	29	21
	t		1.46	1.15	2.87*
	DF		58	58	58

* Indicates significance at the 0.05 level of probability.

APPENDIX C

P Generation Female Body Weights



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APPENDIX D

SUMMARY OF P MALE BODY WEIGHTS (gms)

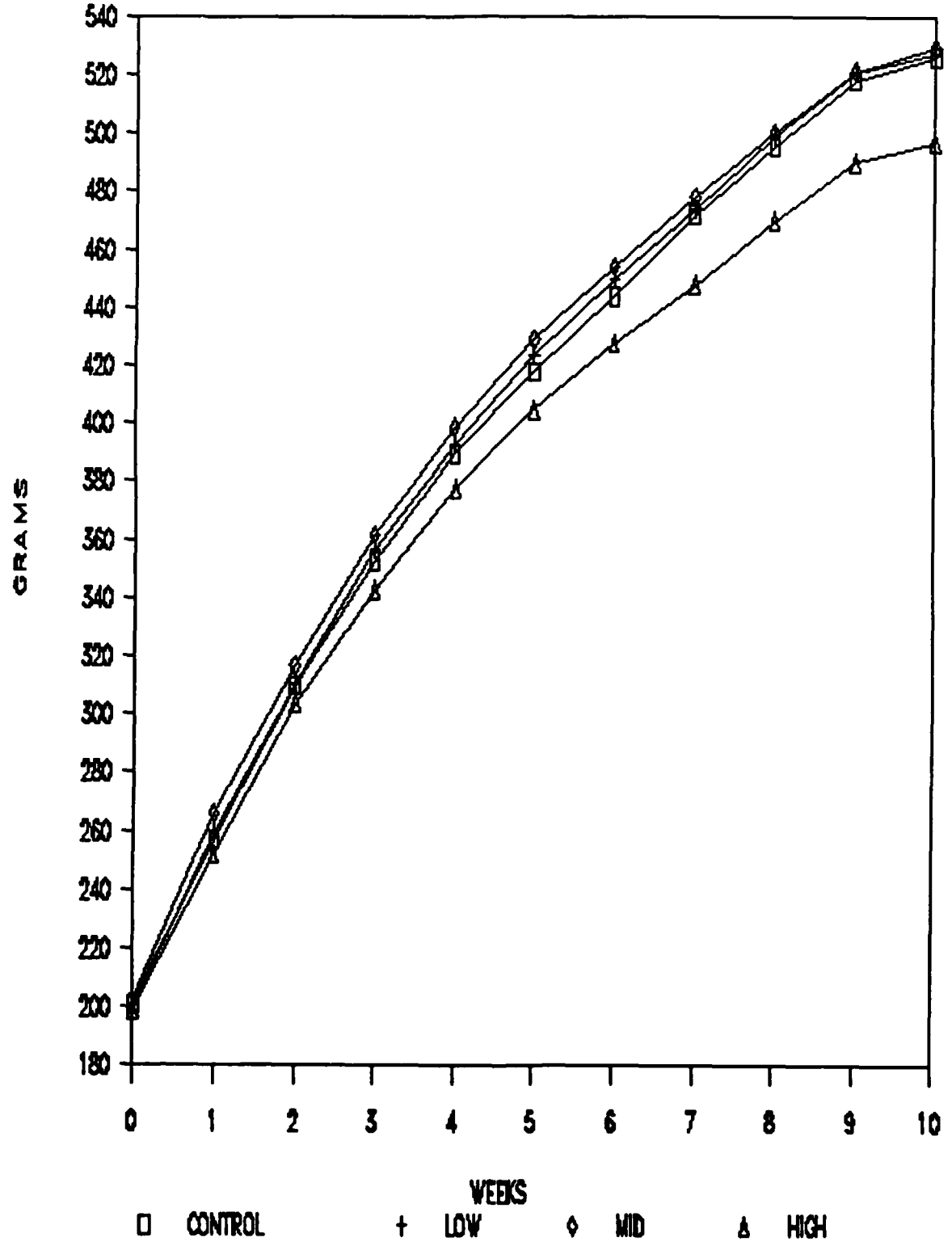
WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
0	x	201	199	202	199
	SD	19	15	16	21
	t		0.58	0.17	0.50
	DF		58	58	58
1	x	257	259	266	252
	SD	27	17	17	28
	t		0.34	1.40	0.80
	DF		58	58	58
2	x	310	311	317	304
	SD	38	20	19	27
	t		0.13	0.91	0.69
	DF		58	58	58
3	x	353	357	362	343
	SD	34	23	21	30
	t		0.43	1.13	1.24
	DF		58	58	58
4	x	390	393	399	378
	SD	40	25	24	33
	t		0.38	1.08	1.29
	DF		58	58	58
5	x	418	424	429	405
	SD	43	28	28	37
	t		0.61	1.14	1.30
	DF		58	58	58
6	x	444	450	454	428
	SD	46	30	33	39
	t		0.62	0.98	1.39
	DF		58	58	58
7	x	472	474	478	448
	SD	51	34	36	43
	t		0.20	0.51	1.91
	DF		58	57	58
8	x	495	499	500	470
	SD	55	39	39	45
	t		0.29	0.36	2.02*
	DF		58	57	58
9	x	518	521	521	490
	SD	59	41	41	47
	t		0.22	0.19	2.07*
	DF		58	57	58
10	x	526	527	529	497
	SD	62	43	41	49
	t		0.12	0.21	2.01*
	DF		58	57	58

* Indicates significance at the 0.05 level of probability.

APPENDIX E

P Generation Male Body Weights

Study #75-51-0497-87, Jan 87 - Feb 88



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APPENDIX F

SUMMARY OF P AVERAGE FEED CONSUMPTION (g/kg/day)

WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
0	male	97.5	96.5	98.5	94.0
	female	92.9	96.1	97.4	95.4
1	male	95.8	95.8	97.9	97.4
	female	94.7	99.4	98.3	99.4
2	male	78.2	81.9	85.3	79.2
	female	81.0	86.8	84.8	84.7
3	male	71.3	73.0	73.2	74.2
	female	77.9	86.1	81.9	80.5
4	male	66.7	67.8	67.3	66.7
	female	78.2	81.4	78.7	77.5
5	male	64.2	61.3	60.6	62.3
	female	72.2	72.4	73.0	71.2
6	male	59.0	58.1	57.5	59.0
	female	71.8	74.8	71.5	70.0
7	male	55.3	55.3	53.4	52.9
	female	68.3	70.7	70.5	66.7
8	male	53.1	53.5	56.1	53.1
	female	63.5	66.3	66.5	64.5
9	male	48.8	49.5	48.8	49.6
	female	56.9	58.2	61.2	62.7
10	male	47.7	48.5	47.7	47.5
	female	58.8	57.5	60.8	60.9

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APPENDIX G

SUMMARY OF P AVERAGE ZNNA CONSUMPTION (mg/kg/day)

WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
1	male	0	48	98	487
	female	0	50	98	497
2	male	0	41	85	396
	female	0	43	85	423
3	male	0	37	73	371
	female	0	43	82	402
4	male	0	34	67	347
	female	0	41	79	388
5	male	0	31	61	312
	female	0	36	73	356
6	male	0	29	58	295
	female	0	37	72	350
7	male	0	28	53	265
	female	0	35	71	333
8	male	0	27	56	266
	female	0	33	67	323
9	male	0	25	49	248
	female	0	29	61	314
10	male	0	24	48	237
	female	0	29	61	305

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APPENDIX H

SUMMARY OF P MATING AND PUP SURVIVABILITY

		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
MATING DAYS	x	2.0	2.9	3.1	2.9
	SD	1.3	3.3	2.9	2.3
GESTATION DURATION	x	22.1	22.2	22.3	22.1
	SD	0.3	0.4	0.6	0.4
FERTILITY INDEX					
No. mating attempts		30	30	30	30
No. observed matings		28	29	29	29
No. positive matings		23	25	23	25
Pregnancies		82%	86%	79%	86%
GESTATION INDEX					
No. pups born		336	330	300	294
No. born alive		330	327	297	291
Percent live litters		100%	100%	100%	100%
VIABILITY INDEX					
No. surviving @ day 4		328	318	297	283
Percent surviving 0-4		98%	98%	99%	97%
LACTATION INDEX					
No. surviving day 4-21 (after standarization)		200	205	187	191
Percent surviving 4-21		100%	100%	100%	99%

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APPENDIX I

SUMMARY OF P (F1) PUPS

		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
LITTER SIZE					
No. birth	x	13.2	12.4	12.4	11.6
	SD	1.8	2.7	3.1	2.9
	t		1.18	1.17	2.30*
	DF		48	47	48
LITTER WEIGHTS					
Birth (grams)	x	83.4	81.1	81.2	70.0
	SD	11.8	15.6	19.5	17.3
	t		0.59	0.48	3.18*
	DF		48	47	48
Weaning (grams)	x	499	506	485	390
	SD	36.7	31.1	59.8	83.7
	t		0.68	1.01	5.98*
	DF		48	47	48
PUP WEIGHTS					
Birth (grams)	x	6.4	6.7	6.6	6.3
	SD	0.5	0.5	0.5	0.6
	t		2.02*	1.64	0.60
	DF		48	47	48
Weaning (grams)	x	62.4	64.3	62.3	50.6
	SD	4.6	4.2	4.2	6.5
	t		1.57	0.05	7.33*
	DF		48	47	48
SEX PROPORTIONS					
Male		49%	51%	51%	48%
Female		51%	49%	49%	52%

* Indicates significance at the 0.05 level of probalility.

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APPENDIX J

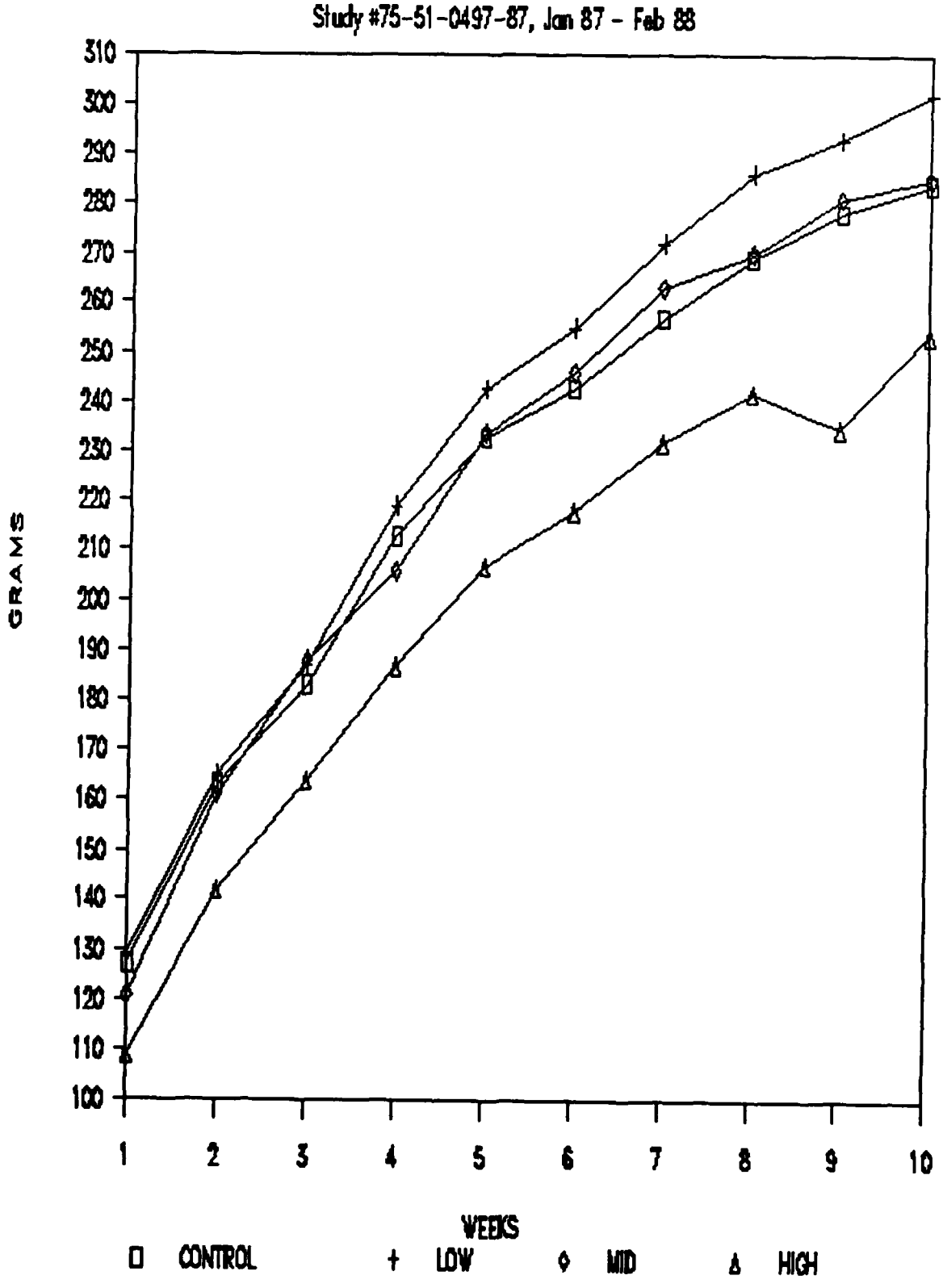
SUMMARY OF F1 FEMALE BODY WEIGHTS (gms)

WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
1	x	127	129	121	109
	SD	11	12	15	10
	t		0.75	1.82	6.44*
	DF		57	58	56
2	x	163	165	161	142
	SD	13	19	17	17
	t		0.51	0.38	5.30*
	DF		58	58	58
3	x	183	187	188	164
	SD	13	17	22	14
	t		1.12	1.11	5.29*
	DF		58	58	58
4	x	213	219	206	187
	SD	17	17	43	15
	t		1.53	0.81	6.26*
	DF		58	58	58
5	x	233	243	234	207
	SD	18	17	22	17
	t		2.10*	0.11	5.75*
	DF		58	58	58
6	x	243	255	246	218
	SD	20	19	24	16
	t		2.49*	0.65	5.39*
	DF		58	58	58
7	x	257	272	263	232
	SD	21	21	30	18
	t		2.70*	0.82	5.00*
	DF		58	58	58
8	x	269	286	270	242
	SD	22	23	26	19
	t		2.82*	0.17	5.02*
	DF		58	57	58
9	x	278	293	281	235
	SD	24	24	29	53
	t		2.49*	0.46	3.95*
	DF		58	57	58
10	x	284	302	285	254
	SD	26	25	28	21
	t		2.69*	0.18	4.83*
	DF		58	57	58

*** Indicates significance at the 0.05 level of probability.**

APPENDIX K

F Generation Female Body Weights



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APPENDIX L

SUMMARY OF F1 MALE BODY WEIGHTS (gms)

WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
1	x	146	151	145	131
	SD	12	17	23	22
	t		1.44	0.25	3.32*
	DF		57	58	57
2	x	210	215	207	187
	SD	16	30	30	30
	t		0.71	0.45	3.78*
	DF		58	58	58
3	x	274	279	250	228
	SD	21	34	36	34
	t		0.65	3.28*	6.40*
	DF		58	58	58
4	x	338	344	326	302
	SD	26	39	46	39
	t		0.79	1.12	4.20*
	DF		58	58	58
5	x	393	407	383	354
	SD	31	43	47	41
	t		1.37	0.98	4.21*
	DF		58	58	58
6	x	430	449	421	385
	SD	39	44	49	42
	t		1.81	0.78	425*
	DF		58	58	58
7	x	471	496	465	425
	SD	41	46	52	45
	t		2.28*	0.50	4.04*
	DF		58	58	58
8	x	504	532	495	453
	SD	48	50	54	49
	t		2.19*	0.67	4.05*
	DF		58	58	58
9	x	530	561	518	477
	SD	52	54	56	51
	t		2.27*	0.84	4.03*
	DF		58	58	58
10	x	557	591	545	499
	SD	57	60	59	55
	t		2.26*	0.81	4.05*
	DF		58	58	58

APPENDIX M

F Generation Male Body Weights

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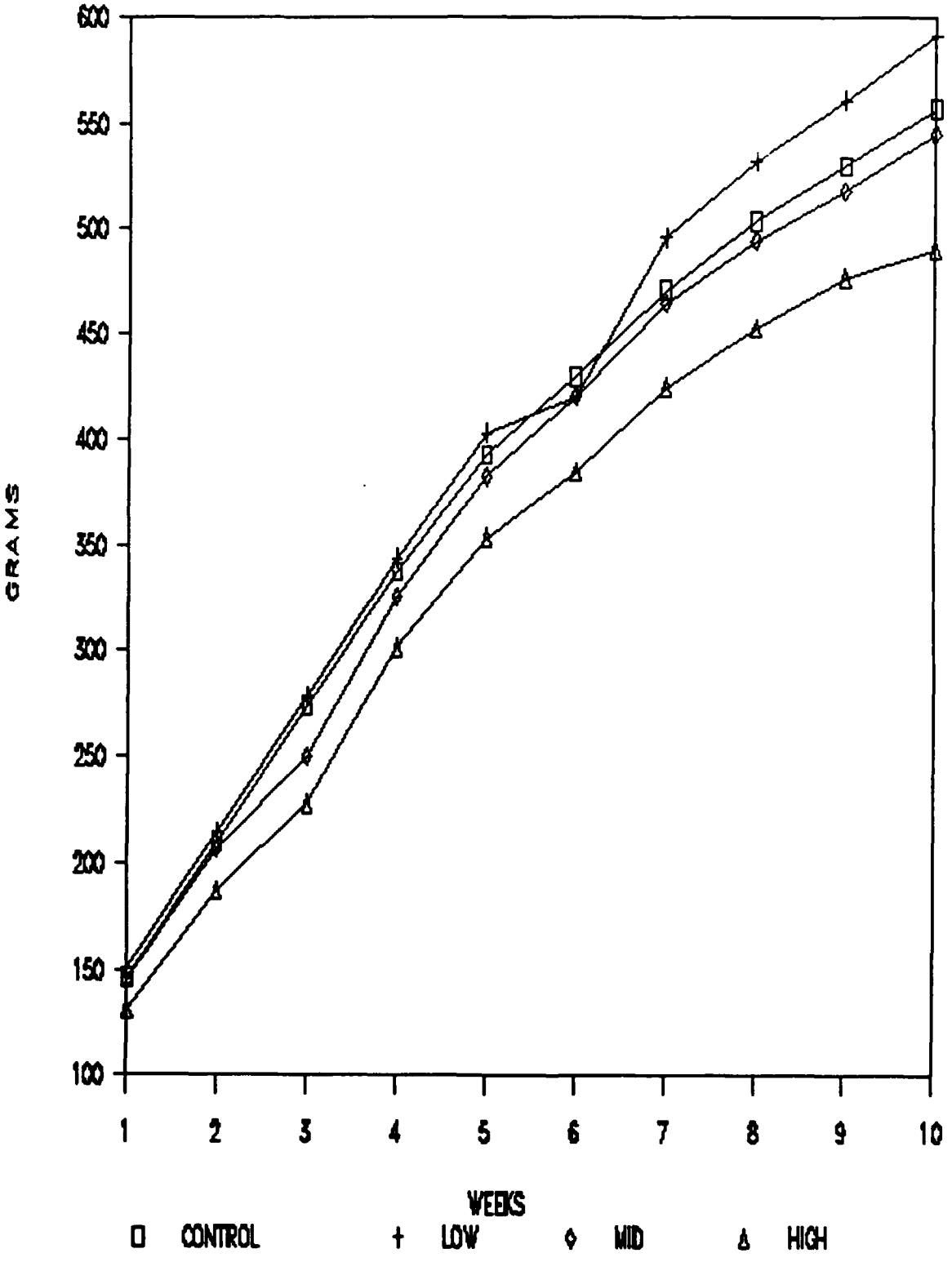

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APPENDIX N

SUMMARY OF F1 AVERAGE FEED CONSUMPTION (g/kg/day)

WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
1	male	125.9	122.4	125.4	127.4
	female	116.4	112.6	120.9	116.2
2	male	107.0	108.3	109.1	111.2
	female	107.6	103.1	98.1	100.7
3	male	98.4	97.7	98.0	100.4
	female	95.6	93.6	91.7	93.2
4	male	85.0	86.2	89.0	91.6
	female	84.5	84.9	84.5	83.4
5	male	72.3	75.3	71.7	73.2
	female	77.5	78.2	77.1	74.8
6	male	62.8	63.3	63.4	64.5
	female	66.5	65.4	66.0	68.5
7	male	60.2	60.2	60.7	60.8
	female	66.4	65.4	65.5	68.0
8	male	56.1	56.0	54.6	56.6
	female	63.8	64.7	61.3	64.7
9	male	64.5	61.6	60.8	63.3
	female	54.0	53.1	52.5	54.2
10	male	48.8	48.5	49.0	51.1
	female	54.9	55.2	55.8	58.7

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APPENDIX O

SUMMARY OF F1 AVERAGE ZNNA CONSUMPTION (mg/kg/day)

WEEK		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
1	male	0	61	125	637
	female	0	56	121	581
2	male	0	54	109	556
	female	0	52	98	504
3	male	0	49	98	502
	female	0	47	92	466
4	male	0	43	89	458
	female	0	42	85	417
5	male	0	38	72	366
	female	0	39	77	374
6	male	0	32	63	322
	female	0	33	66	343
7	male	0	30	61	304
	female	0	33	66	340
8	male	0	28	55	283
	female	0	32	61	324
9	male	0	27	53	271
	female	0	31	61	317
10	male	0	24	49	256
	female	0	28	56	294

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APPENDIX P

SUMMARY OF F1 MATING AND PUP SURVIVABILITY

		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
MATING DAYS					
	x	5.0	5.4	4.8	4.0
	SD	2.9	3.3	3.6	2.1
GESTATION DURATION					
	x	22.2	22.2	22.4	22.2
	SD	0.4	0.7	0.5	0.6
FERTILITY INDEX					
No. mating attempts		30	30	29	30
No. observed matings		20	18	27	29
Percent pregnancies		67%	60%	93%	97%
GESTATION INDEX					
No. pups born		277	263	349	376
No. born alive		271	262	345	371
Percent live litters		100%	100%	100%	97%
VIABILITY INDEX					
No. surviving at day 4		267	257	346	368
Percent surviving 0-4		96%	98%	99%	97%
LACTATION INDEX					
No. surviving day 4-21 (after standarization)		157	143	205	224
Percent surviving 4-21		99%	99%	100%	100%

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APPENDIX Q

SUMMARY OF F1 (F2) PUPS

		CONTROL corn oil	LOW 500ppm	MID 1000ppm	HIGH 5000ppm
LITTER SIZE					
No. birth	x	13.9	14.6	12.9	13.3
	SD	2.4	2.9	3.7	1.9
	t		0.9	1.0	1.0
	DF		36	45	46
LITTER WEIGHTS					
Birth (grams)	x	85.8	92.9	84.0	85.1
	SD	13.6	17.5	23.7	11.6
	t		1.40	0.31	0.21
	DF		36	45	46
Weaning (grams)	x	479	463	444	405
	SD	41.1	45.1	75.4	32.6
	t		1.11	1.88	6.90*
	DF		36	45	46
PUP WEIGHTS					
Birth (grams)	x	6.4	6.4	6.6	6.5
	SD	0.4	0.5	0.4	0.4
	t		1.11	1.28	0.33
	DF		36	45	46
Weaning (grams)	x	61.1	58.3	58.6	50.7
	SD	3.4	4.8	3.7	4.1
	t		2.02	2.28	9.22*
	DF		36	45	46
SEX PROPORTIONS					
Male		50%	48%	47%	51%
Female		50%	52%	53%	49%

* Indicates significance at the 0.05 level of probability.

APPENDIX R

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Division, USAEHA, certifies the following with regard to this study:

a. This study was conducted in accordance with:

(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.

(2) Title 40, Code of Federal Regulations, 1985 rev., Part 160, Good Laboratory Practice Standards.

b. Facilities were periodically inspected during its operational phase to ensure compliance with paragraph a, above.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting this study.

Timothy L. Fisher

TIMOTHY FISHER
Chief, Analytical Quality
Assurance Division